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Research Article

CHLORANTRINIPROLE AND ITS EFFECT ON THE SOIL ECOSYSTEM

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ABSTRACT

In modern agriculture, pesticides are frequently used for increasing the yield by protecting the crops from pests. These pesticides have a negative impact on the soil health and its microbial population. The present investigation was done to evaluate the impact of insecticide *Chlorantraniliprole* on the total microbial populations, enzymatic activity and various physical parameters (pH, electrical conductivity and organic carbon) in *Phaseolus vulgaris* cultivated soils. Soil samples were collected before the insecticide spray and on the 3rd, 10th, 20th and 30th day after the insecticide spray from the *Phaseolus vulgaris* cultivated fields of Singanayakanahalli, Doddaballapur, (Field 1), from fields sprayed with the recommended amount of the pesticide (Field 2) and from field treated with only manure (Field 3) (University of Agricultural Sciences; Bengaluru). Results indicated a significant reduction in the microbial populations and the enzymatic activity. After this initial reduction, the impact of the pesticide became weaker and there was a rise in the microbial population and the enzymatic activity. The values were recorded highest in the 30th day sample. Field 2 and Field 3 soils showed minimum variation with respect to the parameters considered.

Keywords: Aerobic Heterotrophs, Chlorantraniliprole, Enzymatic activity, Phosphate Solubilizers, *Phaseolus vulgaris*.

1. INTRODUCTION

The Pests that attack the crop plants are controlled by using pesticides which are mostly chemicals that have a negative impact on the pests. They can also show an effect on the number and activity of beneficial microbial communities [1]. The pesticides reduce the population of microbial flora in the soil by influencing in various biochemical transformations [2]. Out of the total pesticide applied, only 0.1% reaches the target organism, the rest contaminates the soil [3]. The role of soil microbes in nutrient cycling and decomposition plays an important role in the soil ecosystem [4].

The pesticides may show a short term or long-term effect on the soil microflora. The application of pesticides for a long term affects the microbial biomass, affects the bacterial and fungal activity the soil pH, enzymatic activities and carbon in soil [5]. The long-term use can also contaminate the soil with toxic chemicals.

Chlorantraniliprole (DPX-E2Y45, Rynaxypyr®, Coragen ®), a compound by DuPont is an insecticide belonging to anthranilic diamides class and features a novel mode of action (group 28 in the IRAC classification). It shows a high intrinsic activity towards the target pests, long lasting crop protection, strong ovi-larvicidal and larvicidal properties, low toxicity towards mammals and shows no cross-resistance towards other insecticides. It activates ryanodine receptors (RyRs), thus stimulating the release of intracellular calcium from sarcoplasm reticulum which leads to impaired muscle regulation, paralysis and death [6].

The present study was carried out to evaluate the response of soil microorganisms to indiscriminate use of a pesticide *chlorantraniliprole* applied to legumes *Phaseolus vulgaris* cultivated rain-fed field soils, soil receiving recommended fertilizers and from field receiving only organic manures.

2. MATERIAL AND METHODS

Analysis of soils for selected physical and enzymatic parameters was done before the insecticide spray and on the 3rd, 10th, 20th and 30th day after the insecticide spray. The temperature conditions ranged from 22°C-28°C. The soil collected was of laterite type.

2.1. Collection of Soil Samples

The soil samples were collected from the fields of *Phaseolus vulgaris* sprayed with chlorantraniliprole (field 1) from Singanayakanahalli, Doddaballapur, Bangalore, Karnataka. The soil samples from the fields receiving recommended fertilizer and pesticide (field 2) and field

following organic practices (field 3) for cultivation of *Phaseolus vulgaris* was collected from GKVK, Bangalore. The soil sampling was done using quartet method [7], was air dried at room temperature, sieved through 2 mm sieve to remove stone, powdered in a pestle and mortar and plant debris and stored at 4°C till further analysis.

2.2. Enumeration of Microorganisms

The sieved soil samples (10g) were mixed with 90 ml of sterile saline solution. It was shaken for 30 minutes in a shaker incubator, serially diluted and plated by spread plate method. Serial dilution concentrations 10^4, 10^5, 10^6 were used and following microbial colonies were isolated using the serially diluted solutions [8].

2.3. Enumeration of Total Viable Aerobic Heterotrophic Bacteria:

The plates of Trypticase Soy Agar (TSA) were inoculated and incubated at 37°C for 48 hours after which the plates were examined for growth. The colonies formed were recorded as total viable aerobic heterotrophic bacteria in the sample [9].

2.4. Enumeration of Total Rhizobia

Congo Red Yeast extract mannitol agar (CRYEMA) medium was used for the enumeration of rhizobia. Separately sterilized 1.400 aqueous Congo red solution (10ml) was added to the sterilized solution [10]. The plates were incubated at 25°C up to 7 days. Since rhizobia do not absorb red color of Congo red, the number of colorless colonies represented the number of rhizobia. Rhizobial colonies are characteristically watery on CRYEMA [11].

2.5. Enumeration of Total Phosphate Solubilizers (PSB)

For enumeration of PSB, Pikovskaya's solid medium was used. The number of colonies showing zone of clearance due to solubilization of calcium phosphate in the medium indicated the number of PSB. Well isolated colonies showing zone of clearance around them were counted and noted [12].

2.6. Enumeration of Fungi

Serially diluted aliquots of soil sample were plated by spread plate technique on Potato Dextrose Agar (HiMedia). The plates were incubated at room temperature for 4-7 days [13].

2.7. Enumeration of Azotobacter

Pour plate technique was employed for the isolation of *Azotobacter Sp*. One ml from serially diluted sample was pipetted out and poured in sterile Petri plates followed by 20 ml of sterile Jensen's media (HiMedia). After solidification, the plates were incubated at 37°C for 24-96 hours. After the incubation the brown, glistening, slimy colonies were counted [14].

The colony forming units per gram (CFU/g) of the soil samples were counted. Five replicates were maintained in each case.

2.8. Estimation of Enzyme Activity in the Soil

Urease and alkaline phosphatase activities were estimated following the methods described by Tabatabai and Bremner, 1969 [15].

2.8.1. Urease

Soil (0.1 g) was mixed with 5% aqueous HCl and incubated at 25°C for 24 hours. Urea solution (1ml, 10%) was added and incubated at 37°C for 24 hours. Nessler's reagent was added, and the absorbance was read at 410nm. The urease activity was expressed as amount of urea hydrolyzed per gram of soil sample.

2.8.2. Alkaline Phosphatase

The soil samples (1 g) were incubated with 1 ml disodium phenyl phosphate (10mM) at 37°C for 1 hour on a shaker at 100 rpm. After incubation they were centrifuged at 10,000 rpm for 5 minutes. The supernatant was filtered out and 2 ml of 1 M NaOH was added. PNP (p-nitro phenyl phosphate) produced was measured spectrophotometrically at a wavelength of 410 nm. The results were expressed as μg of p-nitro phenyl phosphate released per gram of dry soil.

2.9. Measurement of Selected Physical Parameters of the soil

The effect of pesticide on physical parameters like pH (Elico make pH meter), electrical conductivity [16] and organic carbon content [17] were measured for the soils collected from all the three parameters under consideration at prescribed intervals i.e., 3rd, 10th, 20th and 30th day after treatment for a period of 4 weeks individually.

2.10. Pesticide Residue Analysis by Using HPLC

The pesticide residue extract obtained from the soil was passed through a florosil column and the eluate was collected. The extract was concentrated in a rotary vacuum evaporator. The residue was dissolved in 1.0ml of methanol (HPLC grade). With Chromatograph obtained, the residue (mg/kg) of pesticides in each

sample was calculated considering the retention time. The results of each sample were analyzed by HPLC [18].

3. RESULTS AND DISCUSSION

It was found that there was a decrease in the microbial count with the application of the insecticide suggesting that the organisms are sensitive to chlorantraniliprole. Soils receiving organic manure (field 3) remained stable in microflora and enzyme activities whereas, soils receiving recommended fertilizer and pesticide application (field 2) showed decreased urease and alkaline phosphatase activities. The maximum decrease in microflora was found immediately after the application of the insecticide and it slowly re-established with the dissipation of the residue.

3.1. Microbial analysis

There was a decrease in the microbial count in field 1 where there was application of the insecticide suggesting that the organisms are sensitive to chlorantraniliprole (Table 1). Soils in field 3 receiving manure (Table 1) and field 2 with recommended amount of fertilizer and pesticide (Table 1) remained almost stable with a slight raise in microfloral population. A similar result was observed by Araujo *et al.,* 2003 who stated that the application of chemicals leads to death of the microbial flora and decrease in the microbe number [19]. Gupta *et al.,* 2000 in their study on rice- wheat cropping system stated that the pesticides have a negative impact on the soil microbes and decreases the average population of different microbial population [20].

Table 1: Analysis of Soil Microflora in the soil from Field 1, Field 2 and Field 3

Sample		Aerobic Heterotrophic Bacteria (CFU X 10^8)	Nitrogen Fixers		PSB (CFU X 10^4)	Fungi (CFUX 10^4)
			Rhizobia (CFUX 10^4)	*Azotobacter* (CFUX 10^4)		
Field 1	Initial Day	9.0±0.05	11.3±0.1	10.0±0.66	12.0±0.32	23.0±0.66
	10th Day	4.0±0.3	9.0±0.03	10.0±0.32	16.0±0.05	24.0±0.05
	20th Day	11.9±0.3	17.8±0.5	12.8±0.2	20.4±0.66	27.7±0.66
	30th Day	16.4±0.03	24.0±0.32	15.2±0.66	27.0±0.03	28.6±0.50
Field 2	Initial Day	33.8±0.03	27.0±0.05	26.0±0.57	22.0±0.32	25.8±0.66
	10th Day	35.2±0.05	27.0±0.02	26.0±0.32	22.0±0.57	23.0±0.32
	20th Day	33.0±0.05	27.6±0.05	26.0±0.32	22.0±0.57	27.0±0.05
	30th Day	33.7±0.03	28.0±0.05	26.0±0.05	22.0±0.32	25.0±0.03
Field 3	Initial Day	24.0±0.32	27.0±0.57	36.8±0	30.2±0.57	28.1±0
	10th Day	29.8±0	28.6±0.32	46.4±0,05	31.0±0.66	28.3±0.03
	20th Day	28.2±0	28.0±0.66	46.0±0.03	30.8±0.32	26.4±0.5
	30th Day	28.4±0	28.0±0	46.0±0	30.8±0	28.4±0.03

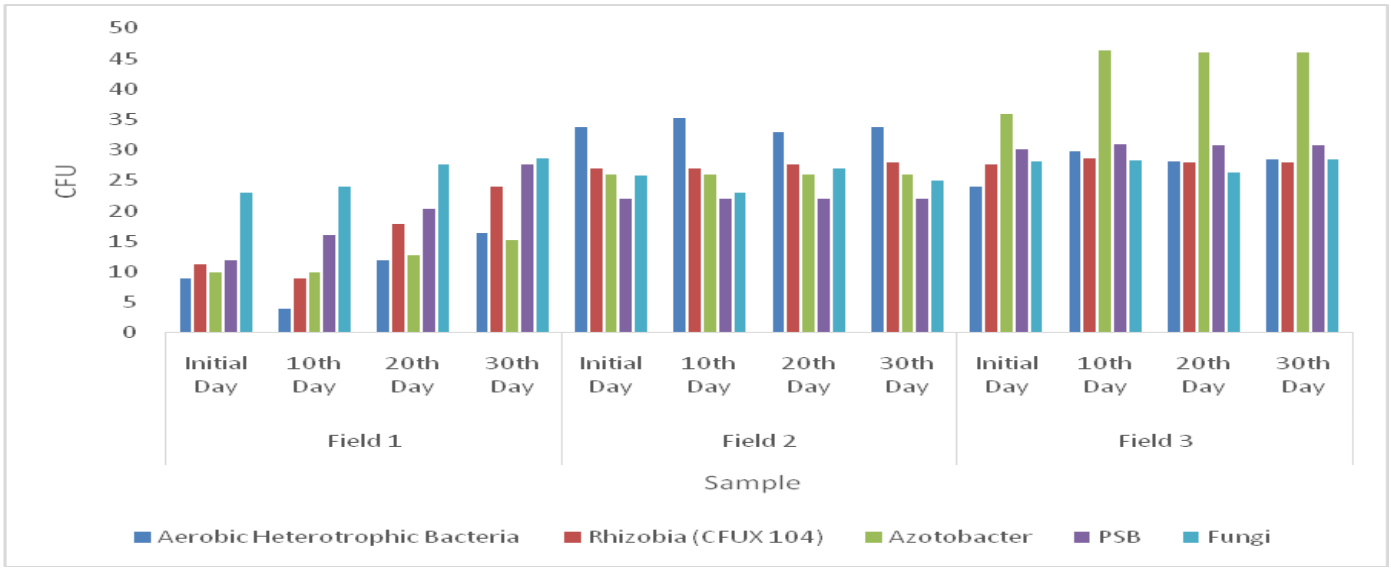

Fig. 1: Analysis of Soil Microflora in The Soil from Field 1, Field 2 and Field 3

3.2. Enzyme activities in the soil

Soils receiving recommended pesticide and manure (Table 2) application showed very little change in the level of urease and alkaline phosphatase activities, whereas the soil sprayed with chlorantraniliprole (Table 2) showed a decrease on the initial days followed by an increase during the 20 and 30th days.

The urease activity was the maximum for field 3 and the least was seen in field 1 with chemical fertilizer (Table 3). A similar result was seen by J. M. Bremner and L. A. Douglas (1971) in their studies on inhibition of urease activity in soil. It was seen that the chemical fertilizers add excess urea into the soil which in turn increases the amount of ammonia. This can lead to the change in soil parameters (acidity, salinity), thus affecting the soil microflora. This in turn can affect the urease activity in the soil [21].

A highest alkaline phosphatase activity was seen in the soils treated with manure from field 3and the least was seen in the soil from field 1. (Table 2). In a study conducted by Sakurai *et al.,* (2008) it was seen that there is a decrease in the alkaline phosphatase activity in the soil treated with chemicals when compared to organic manure [22]. The addition of chemical fertilizer affects the microflora (alkaline phosphatase harboring bacteria) in the soil thus reducing the alkaline phosphatase activity. In a study conducted by Yao *et al.,* 2006 it was seen that the application of pesticides in high concentration had negative impact on soil respiration and phosphatase activity [23].

Table 2: Urease and Alkaline Phosphatase Activity in Soil from Field 1, Field 2(Chlorantraniliprole) and Field 3

Sample		Enzyme Activities	
		Urease (µg of urea hydrolyzed per gram)	Alkaline Phosphatase (µg of p-nitro phenyl phosphate released per gram)
Field 1	Initial Day	30±0.66	142±0.66
	10th Day	40±0	166±0.66
	20th Day	62±0.66	200±0.66
	30th Day	89±0	240±0.66
Field 2	Initial Day	108±0	240±0
	10th Day	110±0.66	234±0
	20th Day	118±0	220±0.66
	30th Day	110±0	220±0.33
Field 3	Initial Day	164±0.33	690±0
	10th Day	164±0	690±0
	20th Day	164±0	690±0.66
	30th Day	164±0.66	690±0.66

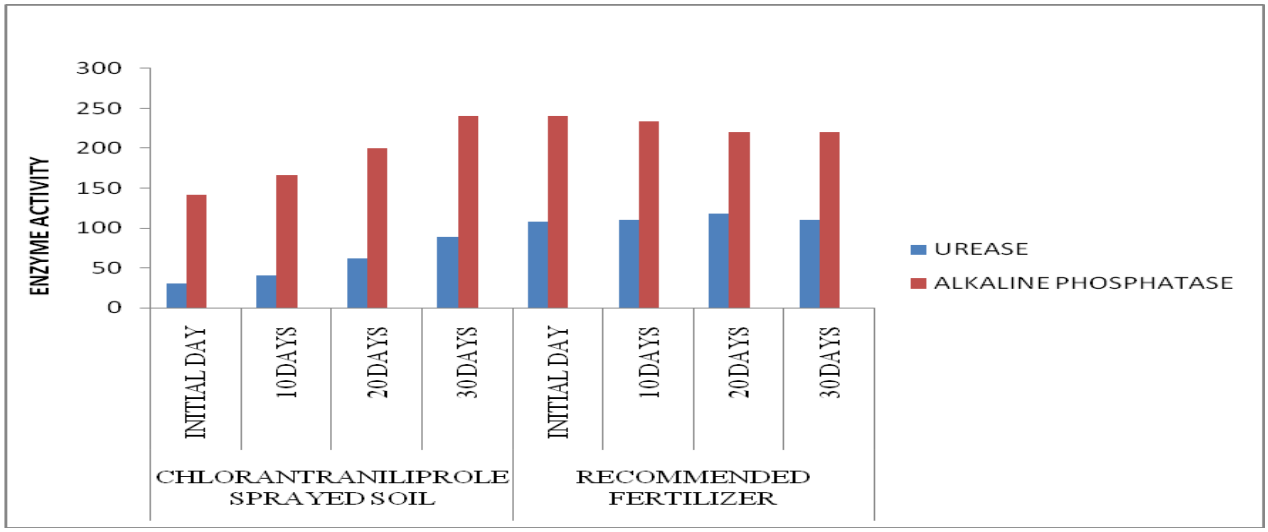

Fig. 2: Urease and Alkaline Phosphatase Activity in Soil from Field 1 (Chlorantraniliprole)

3.3. Physical parameters of the soil

There was a slight change in the pH of the soil from Field 1 when compared to field 2 and field 3 (Table 3). A similar result was seen by Pierre, W.H., 1923, where the addition of chemicals to the soil affected the soil pH that changing the acidity of the soil [24] (Table 3).

The electrical conductivity indicates the dissolved minerals in the soil. The addition of chemical increases the dissolved minerals in the soil. This can be observed in table 3, where there was a slight difference in the electrical conductivity of the soil with uncontrolled pesticide treatment when compared to manure and recommended levels. It was observed by Atafar *et al.,* 2010 that the addition of chemicals to the soil can result in increase of the metal content of the soil [25]. Similar results were observed in the current study. The electrical conductivity of the soil increased in the soil from field 1 (Table 3).

As observed in table 3, there was a decrease in the level of organic carbon in soil from field 1 during the initial days of the spray of pesticide, which increased during the later stages, whereas the organic carbon content remained almost the same in case of soil from field 2 and field 3. These results were similar to the ones obtained by Hati *et al.,* 2008, who stated that adding chemical pesticides reduced the carbon content of the soil [26].

3.4. Pesticide Residue Analysis by Using HPLC

Chlorantraniliprole residue (mg/kg) in the field soils gradually decreased from the initial day to the 20th day. On the 30th day the insecticide had dissipated below LOD (limit of detection) and hence could not be detected by HPLC. Maximum residue was detected on the initial day (Table 4).

Table 3: pH Electrical Conductivity and Organic Carbon Content in Soil from Field 1, Field 2 and Field 3

Sample		pH	Electrical conductivity (µS/cm)	Organic Carbon (g/kg)
Field 1	Initial Day	7.0	5.447	5.447
	10th Day	7.3	6.876	6.876
	20th Day	7.32	6.876	6.876
	30th Day	7.4	6.876	6.876
Field 2	Initial Day	7.18	5.747	5.747
	10th Day	7.18	5.747	5.747
	20th Day	7.18	5.747	5.747
	30th Day	7.18	5.747	5.747
Field 3	Initial Day	7.31	277	4.191
	10th Day	7.31	270	4.180
	20th Day	7.31	272	4.190
	30th Day	7.31	277	4.172

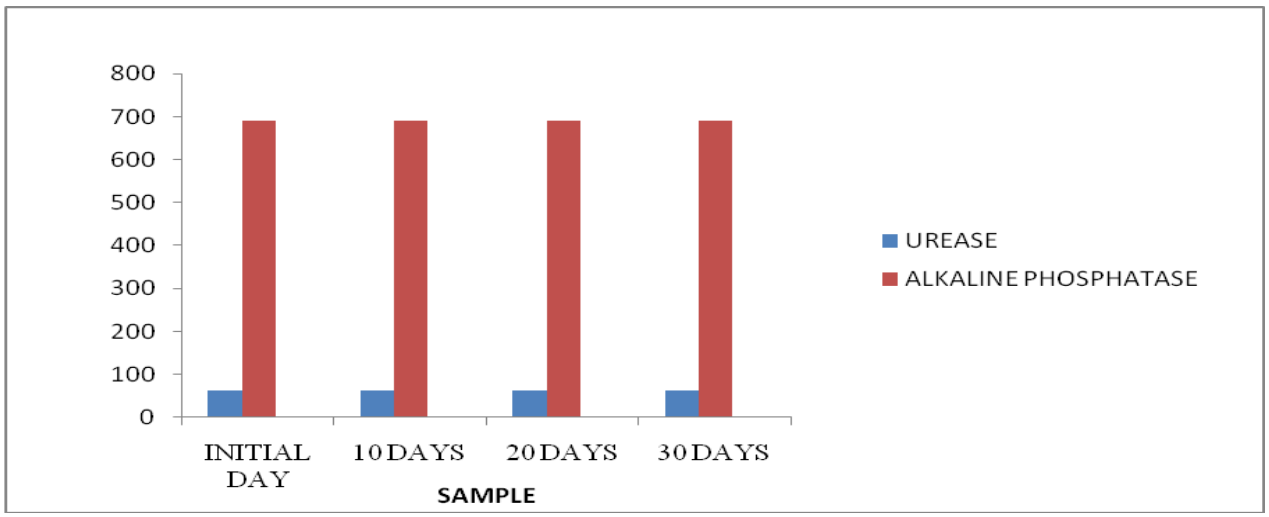

Fig. 3: Urease and Alkaline Phosphatase Activity in Soil from Field 3 (Organic Manure)

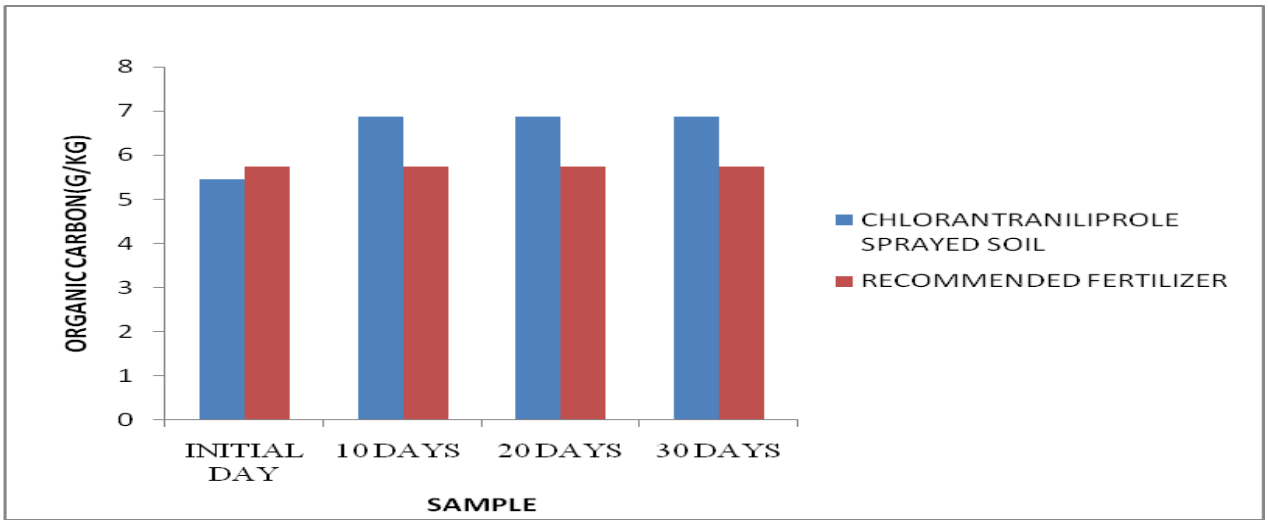

Fig. 4: Organic Carbon Activity in Soil from Field 1 and Filed 2

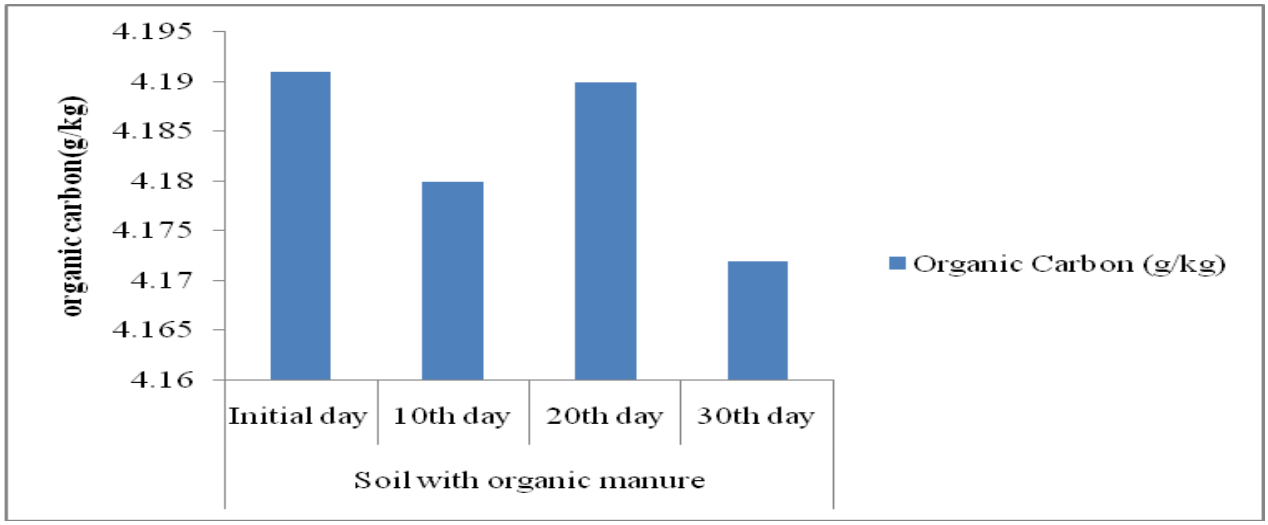

Fig. 5: Organic Carbon Activity in Soil from Field 3

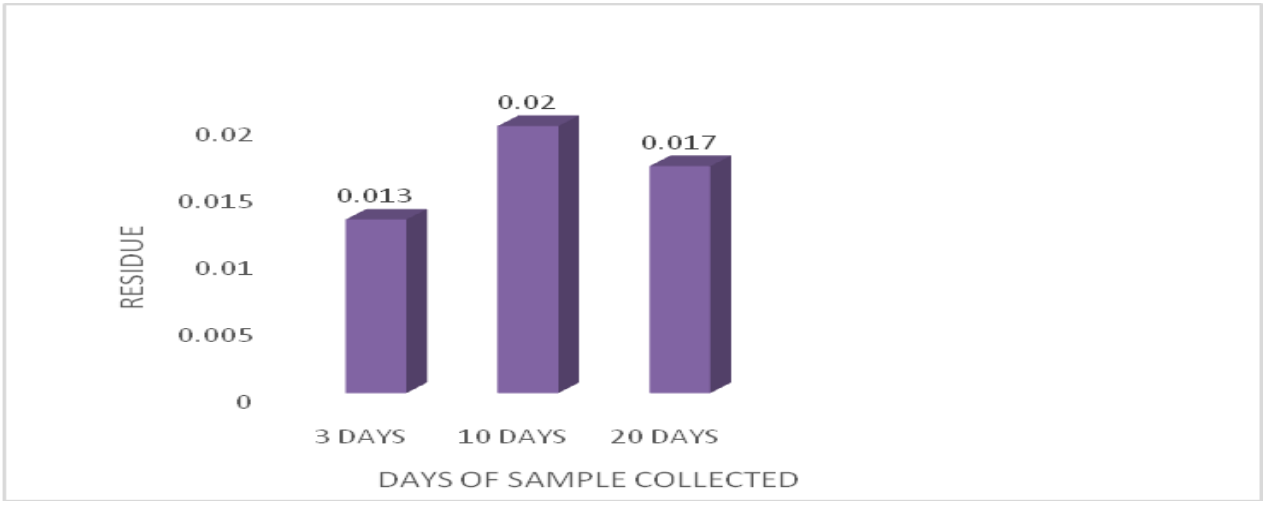

Fig. 6: Pesticide Residue in Soil Sample Collected from Field 1

Table 4: Pesticide Residue in Soil Samples of Field 1

Sample no	Sample	Residue (mg/kg)
1	3 Days	0.013
2	10 Days	0.02
3	20 Days	0.017
4	30 Days	-

4. CONCLUSION

The indiscriminate use of pesticides has become a matter of serious environmental concern altering the soil fertility status, as they affect adversely on soil microorganisms as well as on physicochemical properties of the soil.

Though the efficacy of pesticides in controlling the pest is important, its residual impact should also be considered for environmental safety. The study confirmed that chlorantraniliprole may alter the microbial populations with respect to different days after treatment and thereby affects the soil enzyme activities.

Therefore, there is a need for the advent and use of cheaper, eco-friendly alternatives like application of organic manures, mineral fertilizers and bio-pesticides. This would increase in crop production on judicious use of the known arsenal of agrochemicals as suggested by the integrated pest and nutrient management protocols to restore soil productivity with the activity of soil flora and fauna.

Conflict of interest

None declared

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